Journal of Medical Virology 57:290–297 (1999)

Epitope Mapping of Mouse Monoclonal Antibodies to the ppUL83 Lower Matrix Phosphoprotein of Human Cytomegalovirus

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Of nine mouse monoclonal antibodies (MAbs) directed against the lower matrix protein (pp65; ppUL83) of human cytomegalovirus (HCMV), all immunoprecipitated the 65-kDa protein. Only five were reactive by Western blotting, however, and four of these mapped to linear antigenic epitopes located between amino acids 184–195 (MAb C6), 343–357 (MAb C11), 448–462 (MAb C5), and 448–459 (MAb C13). The epitope specificity of the fifth antibody (MAb C3) and the four that recognised nonlinear sites could not be determined. Competition binding studies using HCMV antigen extracted from productively infected human embryonic lung fibroblasts (HELF), in an enzyme immunoassay (EIA), showed that three of the antibodies reactive with linear epitopes and two of those reactive with conformational epitopes (MAbs C3, C6, C11, C14, and C18), were unique in their binding specificities. MAb C4 competed with MAb C8 and MAb C5 competed with MAb C13 for binding to ppUL83. One of the linear epitopes identified, corresponding to amino acids SAFVFPTKDVAL (MAb C6), was an epitope described previously for $CD8^+$ cytotoxic T lymphocytes. ***J. Med. Virol. 57:290–297, 1999.***

KEY WORDS: epitope mapping; lower matrix protein

INTRODUCTION

The human cytomegalovirus (HCMV) lower matrix protein, ppUL83 (pp65), is a 65-kDa phosphoprotein encoded by the 5′-terminal part of a bicistronic delayed-early 4-kb mRNA [Somogyi et al., 1990; Dal Monte et al., 1996]. ppUL83 is a major constituent of the viral tegument and dense bodies such that human embryonic lung fibroblasts (HELF) infected in vitro with HCMV express detectable amounts of ppUL83 in the cell nucleus prior to the commencement of viral protein synthesis [Grefte et al., 1992]. This represents exogenous protein material that is carried into the cell from the viral inoculum, which is then readily transported to the nucleus mediated by the nuclear targeting signals present within amino acids 402–431 and 537–561 [Schmolke, Drescher, et al., 1995; Gallina et al., 1996]. Nuclear localisation of ppUL83 in polymorphonuclear leukocytes, in the peripheral blood of infected patients, detected by immunocytochemical staining with specific MAbs, is the basis of a sensitive diagnostic test, the so called antigenaemia assay, for same-day detection of viraemia in immunocompromised patients [The et al., 1995].

Functional analysis of ppUL83 has shown that it is dispensable for in vitro replication of HCMV [Schmolke, Kern, et al., 1995]. It is known that pp65 has kinase activity, one role of which is in phosphorylating the viral immediate early (IE) gene products within the infected cell [Roby and Gibson, 1986; Gilbert et al., 1996]. The phosphorylation of these proteins may prevent their degradation and entry to class I major histocompatibility complex (MHC) pathway, which can result in abrogation of their peptide presentation for recognition by cytotoxic T (Tc) cells [reviewed by Riddell and Greenberg, 1997].

Although ppUL83 may interfere with the presentation of other viral proteins, the protein itself is a significant antigen recognised by both humoral and cell-mediated immune responses. The immunodominant nature of this protein is reflected in the detection of specific Tc cells in the peripheral blood of latently infected individuals, in higher frequencies than Tc cells

Grant sponsor: Wellcome Trust; Grant number: 041489/PMG/VW; Grant sponsor: the British Heart Foundation; Grant number: PG/97103.

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Accepted 16 August 1998

specific for any other viral component including the IE proteins [Wills et al., 1996]. The recognition specificities of Tc cells for ppUL83 have been mapped to nine different peptide epitopes and there is evidence in some individuals that the cytotoxic response is highly focused, with cells recognising a single peptide [Wills et al., 1996]. Studies of the humoral immune response to ppUL83, by immunoblotting of purified virions or extracts of infected cells, have shown ppUL83 to be one of the most abundant of the viral proteins recognised by more than 50% of HCMV antibody-positive human sera [Landini et al., 1990]. B-cell epitope mapping of ppUL83 has been attempted previously mainly for the purpose of producing recombinant expressed proteins or synthetic peptides for use in serological assays [Landini et al., 1990]. Such studies have suggested that humoral immune responses to ppUL83 are mainly against conformational epitopes [van Zanten et al., 1995]. A discontinuous epitope involving residues 208–216 and 280–285 has been described by Ohlin et al. [1991]. Linear B cell epitopes have also been characterised. These include regions spanning residues 283–288 [Ohlin et al., 1991], 297–458, 401–470 [Landini et al., 1990], 401–426 [Plachter et al., 1990], 184–379, and 410–427 [Ohlin et al.,1995].

In the present study, mouse monoclonal antibodies (MAbs) specific for ppUL83 have been used for further mapping of B-cell linear and conformational epitopes in ppUL83. New linear epitopes have been defined, one of which, corresponding to amino acids 184–195, has been reported previously to be a target for Tc cells in healthy subjects of HLA B35 haplotype [Wills et al., 1996].

MATERIALS AND METHODS

Virus Strains

The AD169 strain of HCMV was cultured in monolayers of mycoplasma-free HELF. When extensive cytopathic effect (CPE) was observed the cells were frozen at −70°C, thawed at 37°C, centrifuged at 3,000 × *g* to remove cell debris, and the supernate stored at −70°C. Control antigen was prepared in the same way, but from mock-infected HELF.

Preparation of MAbs

As described by Baboonian et al. [1989], adult Balb/c mice were inoculated intraperitoneally, on four occasions, with the AD169 strain of HCMV. Spleen cells, harvested 3 days after the last injection, were fused with the myeloma cell line NS1 and hybrid cells were selected using medium containing hypoxanthine, aminopterin, and thymidine. Cultures producing specific antibody, by enzyme immunoassay (EIA), were cloned by limiting dilution and used to prepare mouse ascitic fluid or bulk culture supernate.

Antibody Purification Using Affinity Columns

Culture supernates (250 ml) were concentrated 25-fold by ultrafiltration (Centriprep concentrators, Amicon), dialysed against 20 mM sodium phosphate buffer pH 7.0, then purified on Hi Trap™ affinity protein G columns (Pharmacia) according to the manufacturer's instructions. The peak fractions of mouse Ig, were pooled and reconcentrated to 2 ml, dialysed against 0.1 M sodium phosphate buffer pH 7.2 and, as required, were biotinylated with biotinyl-E-amino caproic acid *N*-hydroxysuccinimide ester (Sigma) according the manufacturer's instructions. The biotinylated antibodies were purified by gel filtration (Sigma) and quantitated by measurement of absorbance at 280 nm.

EIA

Antibody to HCMV was determined by EIA, using alkali-extracted antigen from HCMV AD169 infected HELF. Polystyrene plates (Nunc; Gibco BRL) were coated with the viral or the control antigen (the latter as extracts of uninfected cells) at the optimum dilution in bicarbonate buffer, pH 9.6, at 4°C overnight. The plates were washed thoroughly in phosphate buffered saline containing 0.05% Tween 20 (PBST) tapped dry and stored at −70°C. Samples to be tested for antibody were used either as undiluted cell culture supernate or a 100-fold dilution of mouse ascitic fluid in PBST. These were incubated in the virus antigen-coated and the control antigen-coated strips for 1 hr at 37°C, followed by washing in PBST then incubation with alkaline phosphatase conjugated anti-mouse antibodies (Sigma). *p*-Nitrophenyl phosphate was subsequently used as the substrate. Characterisation of individual MAbs in terms of IgG subclass and light chain specificity was carried out by EIA using solid phase HCMV antigen and either subclass-specific or light-chain-specific rabbit anti-mouse antibodies (Sigma).

In the competition EIA, strips coated with the virus antigen and with the control antigen were incubated with serial four-fold dilutions of unlabelled MAb, in the form of affinity purified, concentrated cell culture supernate, at 37°C for 1 hr. The plates were washed with PBST, then incubated for 1 hr at 37°C with a predetermined optimum dilution of biotin-labelled MAb followed by incubation with Extravidin-conjugated alkaline phosphatase. The binding of the labelled MAb was quantified using the chromogen, *p*-nitrophenyl phosphate.

Epitope Mapping

Nonapeptides corresponding to the entire sequence of ppUL83 and overlapping each other by six amino acids were synthesised using a multipin system (Chiron Mimotopes Pty Ltd) that was designed to fit into a microtitre plate. The pins were initially coated in PBS containing 1% w/v bovine serum albumin (BSA) and 1% ovalbumin for 1 hr, at 4°C. The pins were then placed in microtitre plates containing MAb in the form of buffered (25 mM HEPES) neat cell culture supernate or a 1:400 dilution of ascitic fluid. After an overnight incubation at 4°C, the pins were treated with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (Amersham). Binding of the antibody to specific peptides was assessed using the substrate 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulphonic acid) diammonium salt (Sigma).

Immunoblotting

Polyacrylamide gel electrophoresis of proteins from HELF infected 7 days earlier with 0.5 pfu per cell of HCMV was carried out, under reducing conditions, using a 10% gel. Following electrophoretic transfer to nitrocellulose filters, these were treated with PBS containing 2% dried milk powder prior to incubation with the MAbs at room temperature for 1 hr. The bands were visualised with alkaline phosphatase conjugated rabbit anti-mouse antibodies using 5-bromo-4-chloro-3-indolyl phosphate (Sigma) as the substrate. A MAb directed against a varicella zoster virus (VZV) nuclear protein (a generous gift from Dr. David Harper) was used as a negative control antibody.

Radioimmune Precipitation (RIP) Test

Confluent HELF monolayers were infected with HCMV AD169 at an input multiplicity of 0.5 pfu per cell. When extensive CPE was observed, usually after 7 days, the cells were placed in methionine-free Eagle's MEM (Gibco BRL) for 2 hr at 37°C, then labelled with 100 μCi per milliliter of ^{35}S methionine (ICN Flow) for the same period of time. The cells were washed with PBS and extracted by lysis into 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% v/v nonidet P40, 1% sodium desoxycholate, 1 μg/ml aprotonin, 1 mM ethylenediamine tetraacetic acid (EDTA), and 20 μg/ml phenyl methyl sulphonyl fluoride. After incubation for 30 min on ice, the lysate was transferred into lysis buffer containing 2 mg/ml of BSA and cell debris was removed by centrifugation. Clear extracts were treated with normal rabbit serum (100 μl of serum per milliliter of lysate) for 1 hr on ice, then ultracentrifuged at 70,000 × *g* for 1 hr at 4°C. The clarified supernates were then adsorbed twice with equal volumes of protein A-bearing *Staphylococcus aureus* cell suspension (Pansorbin; Sigma), for 30 min on ice. Uninfected HELF were processed, for control antigen preparation, in an identical manner.

To carry out the RIP test, 350–500 μl of MAb in the form of culture supernate or 5 μl of ascitic fluid was mixed with the ^{35}S methionine-labelled antigen containing 500,000 cpm and incubated at 4°C for 1 hr, after which 50 μl of protein A-Sepharose (Sigma) was added and the mixture was further incubated for 1 hr on ice with continuous agitation. The precipitates were washed with lysis buffer containing 1% BSA followed by two washes with buffer alone and one wash with 50 mM Tris-HCl containing 0.1% nonidet P40. The precipitates were dissociated by boiling in 60 mM Tris-HCl (pH 6.8) containing 2% sodium dodecyl sulphate and 5% 2-mercaptoethanol for 5 min, then run on a 10% polyacrylamide gel followed by processing for fluorography. A neutralising MAb C2, directed against the glycoprotein-H (gH) of HCMV was used in this experiment as a control reagent [Baboonian et al., 1989].

RESULTS

Immunoblotting and Immunoprecipitation

Nine MAbs (designated MAbs C3, C4, C5, C6, C8, C11, C13, C14, and C18) were investigated. All except C13 were used as cell culture supernates; MAb C13 was available as ascitic fluid. All antibodies were IgG2a of κ light chain specificity, with the exception of C4, which was IgG1. The MAbs did not neutralise infectivity of HCMV when tested alone or in the presence of complement (data not shown). All nine antibodies were positive by immunofluorescence against cells infected with HCMV. Positive immunofluorescence was also observed against cells infected with a defective adenovirus expressing the ppUL83 gene (details to be published separately).

Inmmunoprecipitations using extracts of infected HELF showed that the antibodies were reactive with a major protein of 65 kDa and two minor polypeptides of 50 kDa and 52 kDa (Fig 1A). Our findings are consistent with the presence of the translated product of a shorter mRNA reported by Somogyi et al. [1990]. The initiation site for this message resides 97 bp downstream of that of ppUL83. The HCMV gH-specific MAb precipitated a protein of approximately 86 kDa, corresponding to the estimated molecular weight of this protein. By immunoblotting, MAbs C3, C5, C6, C11, and C13 reacted with a 65-kDa protein and showed binding to an additional polypeptide of 23 kDa, a possible degradation product of ppUL83 (Fig. 1B). Antibodies C4, C8, C14, and C18 showed no reactivity in this assay, suggesting that they may be directed against nonlinear epitopes. No reactivity was observed with a VZV-specific MAb reactive with a viral nuclear protein. None of the antibodies reacted with uninfected HELF either by RIP or immunoblotting.

Pepscan Analysis

Each MAb was tested by EIA against 185 overlapping nonapeptides corresponding to the entire amino acid sequence of ppUL83. MAbs C5, C6, and C11 each reacted with a different discrete linear epitope as denoted by the detection of reactivity involving a small number of adjacent peptides (Fig. 2). The epitope for MAb C5 was localised to the amino acid sequence SGVMTRGRLKAESTV (residues 448–462), with the most efficient binding to the MTRGRLKAE region; that for MAb C6 was confined within SAFVFPTKDVAL (residues 184–195), with maximum binding at VFPTKDVAL, whereas MAb C11 reacted with amino acids DPVAALFFFDIDLLL (343–357) with peak binding at AALFFFDID (Table I). MAb C13, which was in the form of mouse ascitic fluid, showed binding with three separate regions in the amino acid sequence, the most reactive being the same sequence as that of MAb C5, namely SGVMTRGRLKAE (amino acids 448–459). The other two regions reactive with MAb C13 (peptides 86–88 and 147–149) were also recognised by normal mouse serum in the same assay and were hence regarded as nonspecific. The epitope specificities of the

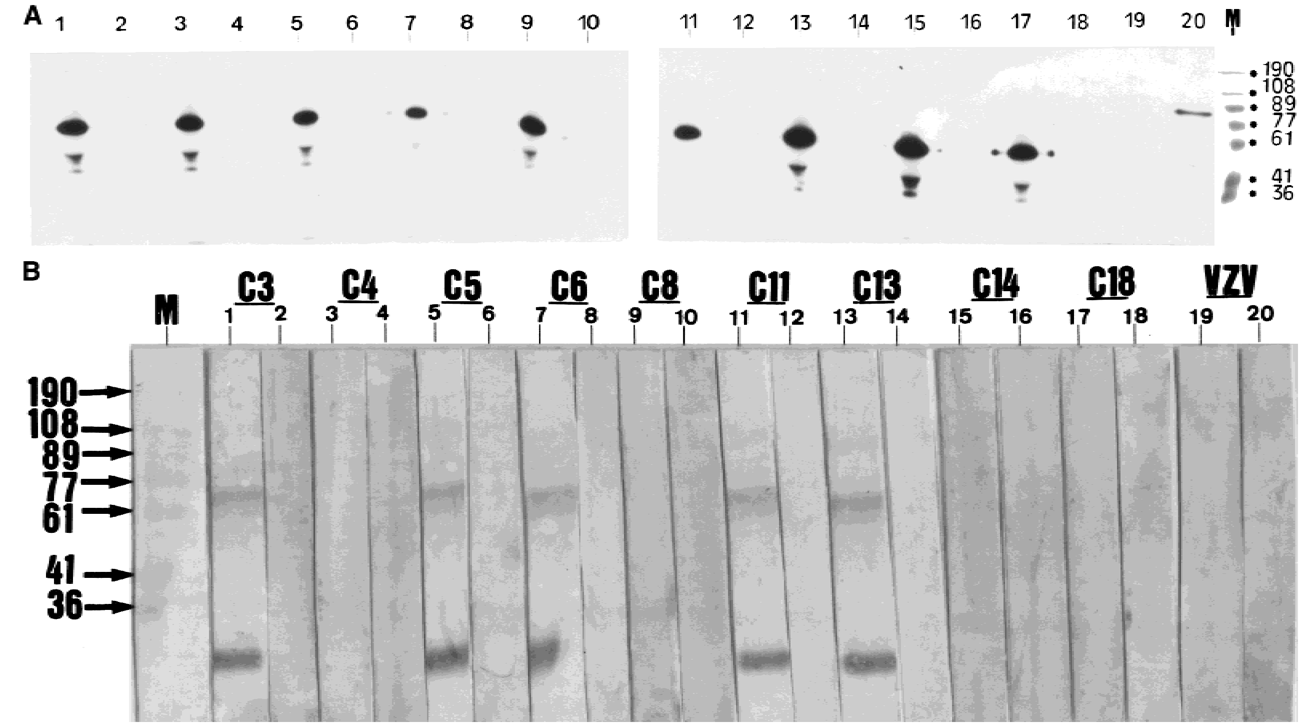

Fig. 1. **A:** Autoradiography of ^{35}S-methionine-labelled human cytomegalovirus (HCMV) antigens precipitated with monoclonal antibodies (MAbs) C3, C4, C5, C6, C8, C11, C13, C14, and C18 (lanes 1, 3, 5, 7, 9, 11, 13, 15, and 17, respectively). MAb C2 directed against glycoprotein-H (gH) of HCMV was used as control (lane 20). Uninfected human embryonic lung fibroblasts (HELF) were used as control antigen (lanes 2, 4, 6, 8, 10, 12, 14, 16, 18, 19). M, molecular weight markers (SDS-7B Sigma); sizes are given in kilodaltons. **B:** Detection of ppUL83 by immunoblotting, using MAbs C3, C4, C5, C6, C8, C11, C13, C14, and C18 (lanes 1, 3, 5, 7, 9, 11, 13, 15, and 17, respectively). A MAb to varicella zoster virus (VZV) was used as control (lane 19). Uninfected HELF were used as control antigen (lanes 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20). M, molecular weight markers (SDS-7B Sigma); sizes are given in kilodaltons.

remaining MAbs, namely; C3, C4, C8, C14, and C18, could not be determined because they were not reactive by Pepscan analysis.

Competition Binding Analysis

MAb in the form of cell culture supernates were prepared by affinity chromatography using protein G columns and labelled with biotin as described in Materials and Methods. Each labelled MAb was used in competition EIA experiments against the homologous and heterologous unlabelled MAb including MAb C13, which was in the form of mouse ascitic fluid and could not be purified for biotinylation. EIA plates coated with viral or control antigen were preincubated with serial four-fold dilutions of each unlabelled MAb and carefully washed. Thereafter the plates were tested again to see if the binding of the unlabelled MAb prevented the subsequent binding of labelled MAbs. The binding of each of the labelled MAbs was inhibited by preincubation of the antigen-coated EIA plates with the homologous unlabelled antibody. In addition, MAbs C5 and C13 competed with one another for binding the HCMV antigen in the EIA; similarly, MAb C8 inhibited the binding of labelled MAb C4 but not any of the other MAbs (Table II). The remaining five antibodies (C3, C6, C11, C14, and C18) showed no heterologous competition in this assay.

These results confirmed that there were MAbs reactive with four linear epitopes whose sequence was identifiable by Pepscan analysis. A fifth MAb (C3) was also reactive with a linear epitope, albeit its sequence could not be identified by the Pepscan method. No competition was detected between the five MAbs directed against linear and the four that were reactive against conformational, nonlinear epitopes.

DISCUSSION

The lower matrix phosphoprotein of HCMV is a major structural component of virions and dense bodies and is a significant antigen in the induction of specific antibodies and, more importantly, of cytotoxic T-cell responses. Previous studies have identified both linear and conformational B-cell epitopes on this protein. Characterisation of nine MAbs in the present study revealed further information on the antigenic structure of ppUL83.

Radioimmunoprecipitation showed that all the antibodies were reactive with a 65-kDa viral protein. Proteins of smaller molecular weight consistent with the 52-kDa and 50-kDa polypeptides described by Britt and Vugler [1987] were also observed. The 52-kDa polypeptide may represent the product of a second mRNA, which Somogyi et al. [1990] reported to be transcribed from the same region of the HCMV genome.

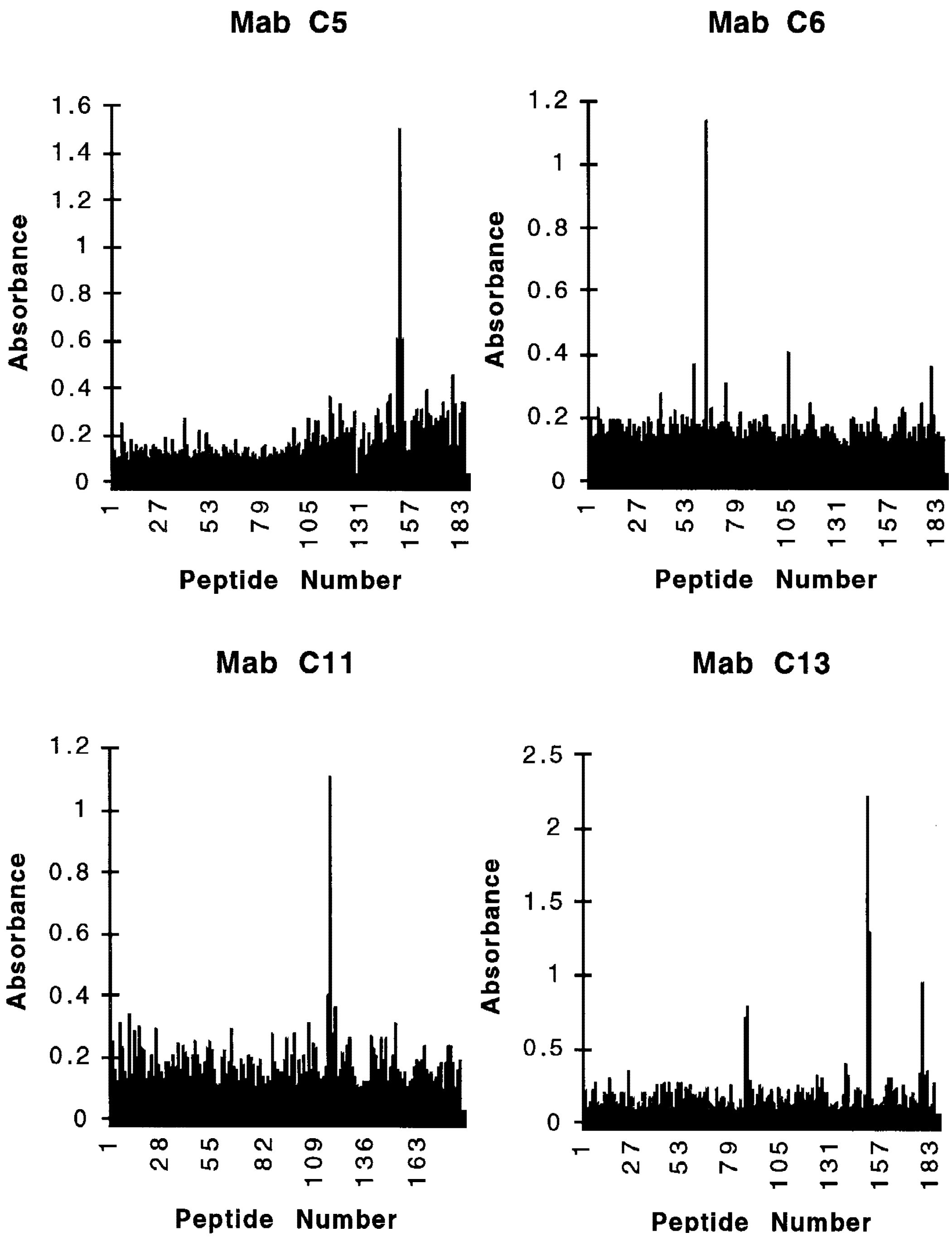

Fig. 2. Binding of monoclonal antibodies (MAbs) to solid phase overlapping nonapeptides using an enzyme immunoassay (EIA). Absorbance values at 405 nm are shown. Residues are numbered according to Ruger et al. [1987].

The initiation site for the second message is thought to be 97 bp downstream of that of ppUL83. It is also likely that a single transcript of this gene may be translated from the AUG at nucleotide positions 341 or 665 [Somogyi et al., 1990]. As all antibodies reacted with the smaller polypeptides, it is possible that the epitope specificity of these was confined to the region represented either by the short transcript or the translated product from the AUG site at nucleotide 665. Immunoblot analysis showed that five of the nine antibodies (namely, MAbs C3, C5, C6, C11, and C13) also reacted with a 65-kDa molecular weight protein, suggesting that they recognised linear epitopes. MAbs C4, C8, C14 and C18 were nonreactive by immunoblotting and therefore were likely to be directed against discontinuous epitopes on this molecule. Strong reactivity was also observed, by immunoblotting, with rapidly migrating polypeptides of approximately 23 kDa. The lower molecular weight band is likely to represent degradation products of ppUL83 co-migrating closely on the

TABLE I. Epitope Sequences Recognised by Monoclonal Antibodies

MAb	Peptide	Amino acid	Amino acid sequence
C5	150–152	448–462	SGVMTRGRL MTRGRLKAE GRLKAESTV
C6	62–63	184–195	SAFVFPTKD VFPTKDVAL
C11	115–117	343–357	DPVAALFFF AALFFFDID FFFDIDLLL
C13	150–151	448–459	SGVMTRGRL MTRGRLKAE

Sequences giving maximum reactivity are underlined. MAb, monoclonal antibody.

TABLE II. Competition Binding Assays

Labelled MAb	Unlabelled MAb								
	C14	C8	C4	C18	C3	C11	C6	C5	C13
C14	+								
C8	−	+							
C4	−	+	+						
C18	−	−	−	+					
C3	−	−	−	−	+				
C11	−	−	−	−	−	+			
C6	−	−	−	−	−	−	+		
C5	−	−	−	−	−	−	−	+	+

MAb, monoclonal antibody. Each MAb was biotinylated and reacted in a competition EIA against unlabelled homologous and heterologous antibodies. MAb 13 was not biotinylated as it was only available in the form of ascitic fluid and it could not be adequately purified. It was therefore used as unlabelled competing Mab against all others.
+, Competition between unlabelled and labelled Mab for binding to HCMV AD169 ppUL83.

polyacrylamide gel. Changes in the preparation and processing of the antigen prior to immunoblotting did not eliminate this band.

Analysis of the B-cell antigenic determinants of the lower matrix protein using overlapping synthetic peptides covering the entire amino acid sequence of the molecule yielded positive results only with MAbs shown to be specific for linear determinants by immunoblotting. The location of these linear epitopes were compared with epitopes reported previously by other workers (Fig. 3).

A 12-amino acid long sequence of the molecule corresponding to positions 184–195 contained the epitope reactive with MAb C6. This region of the ppUL83 molecule has been studied previously by Ohlin et al. [1995], who found that a human MAb bound to peptides corresponding to amino acids 184–379. Analysis of the hydrophilicity profile of the amino acid sequence containing the epitope for MAb C6 showed that this sequence is part hydrophobic and part hydrophilic. Closer inspection of the antibody binding to this region showed that reaction to the second peptide covering the hydrophilic stretch of the molecule was higher. We were also able to better define a second epitope within the region studied by Ohlin et al. [1995]. The linear epitope for MAb C11 corresponded to amino acids 343–357, with the strongest binding to amino acids AALFFFDID. This epitope is located in a hydrophobic part of the ppUL83 molecule. Previous studies by Landini et al. [1991] on another HCMV protein, pp150, have shown that B-cell epitopes are not always located in hydrophilic portions of proteins and that information on the secondary structure of a peptide is not enough for predicting immunogenicity.

Two MAbs, C5 and C13, both reacted with the same or closely related epitopes, which corresponded to the amino acids 448–462 in a hydrophilic region of the molecule, with predicted alpha helical structure [Pande et al., 1990, 1991]. MAb C13 showed higher binding to the amino acid sequence SGVMTRGRLKAE, whereas MAb C5 bound more strongly to the sequence MTRGRLKAE. Landini et al. [1990] and Plachter et al. [1990] have previously studied this region of ppUL83 and shown that fusion peptides covering amino acid sequences 297–458 and 401–470 are reactive with human sera by immunoblotting and EIA. Binding of MAbs C13 and C5 to this area confirmed the presence of B-cell epitopes in this part of the molecule. We were able to show the precise location of another epitope within the same region, corresponding to amino acids 343–357. This section of the molecule, strongly reactive with MAb C11, is a hydrophobic area of ppUL83.

MAbs directed against ppUL83 have been described previously [Britt and Vugler 1987; Ohlin et al., 1991; Gerna et al., 1992; La Fauci et al., 1994] and used extensively in the detection of antigenaemia [The et al., 1995; Sharma et al., 1997; St. George and Rinaldo 1997], but the epitope specificity in many of these is conformational rather than linear. For diagnostic purposes, such as the detection of antigenaemia, the availability of MAbs to a range of different antigenic sites is advantageous in terms of improving sensitivity because pooling of such MAbs may have an additive effect on the intensity of the signal produced in immunofluorescence tests. Use of single antibodies in antigenaemia assays risks the possibility of false-negative results should strain variation in the clinical isolate be sufficient to alter the epitope such as to eliminate antibody binding altogether. Restriction enzyme analysis of the genomic region of the ppUL83 in clinical isolates of HCMV shows minor differences [Chandler and McDougall 1986; Klages et al., 1989], albeit these differences have not been reported to affect antibody binding.

An important finding in the present study was of a linear B-cell epitope identical in sequence to an epitope that is a major target for Tc cells in an individual of HLA B35 haplotype [Wills et al., 1996]. MAb C6 was reactive with the sequence SAFVFPTKDVAL (amino acids 184–195), which is present in two peptides (amino acids 181–195 and 187–201) accounting for 29% of $CD8^+$ Tc cells specific for HCMV in a donor studied by Wills et al. [1996]. The presence of antibodies to T cell epitopes may be significant in the maintenance of virus persistence. Indeed, Charpentier et al. [1987] have shown that Tc cell response was blocked with HCMV-specific MAbs. Studies on chronic hepatitis B virus infections have shown that T-cell cytolysis is

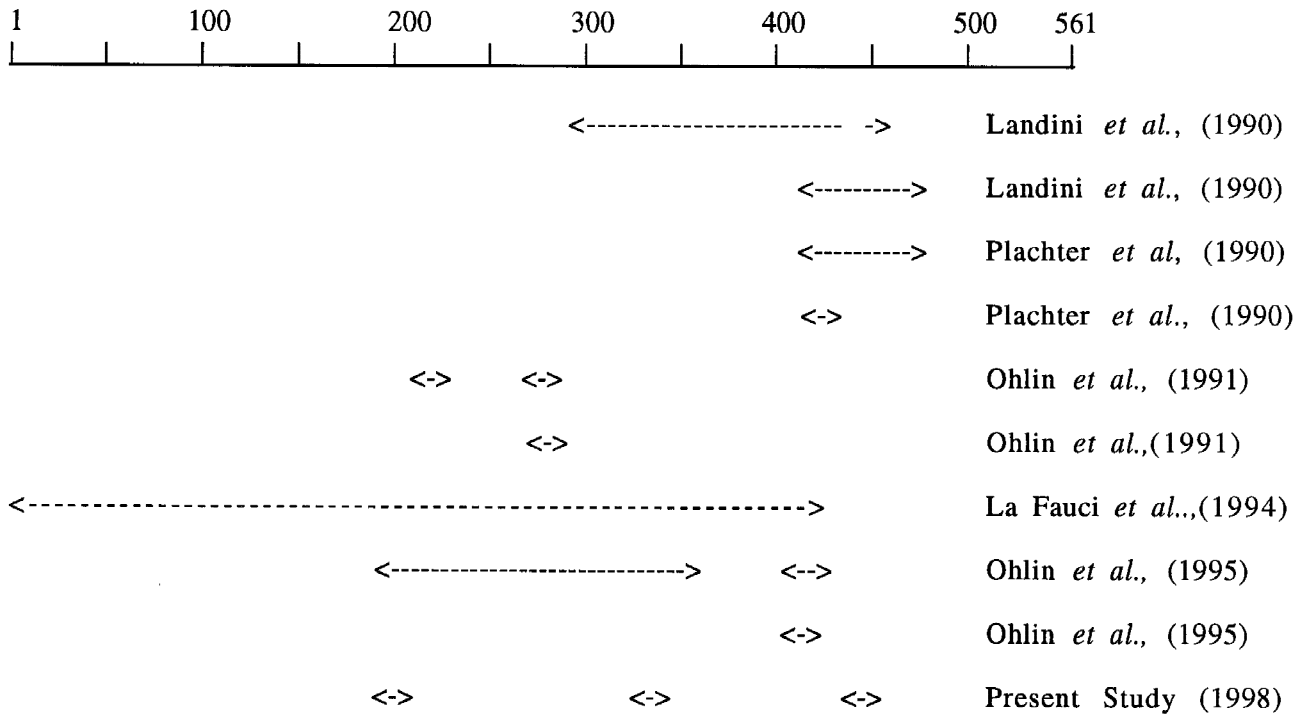

Fig. 3. Regions of ppUL83 identified as containing B-cell epitopes. The positions of peptides are shown against a scale representing the 561-amino acid sequence of this protein.

modulated by anti-hepatitis B virus core antibodies, which bind to infected hepatocytes [Naumov et al., 1984]. The presence of antibodies that react with prominent T-cell epitopes may hinder lysis of infected cells expressing such epitopes, and play a part in immune evasion and prolonged viral excretion or persistence.

ACKNOWLEDGMENTS

This research was supported by grants awarded by the Wellcome Trust (041489/PMG/VW) and the British Heart Foundation (PG/97103). We would like to dedicate this work to the memory of the late Dr. James Clive Booth, who died shortly after the completion of the manuscript.

REFERENCES

Baboonian C, Blake K, Booth JC, Wiblin CN. 1989. Complement-independent neutralising monoclonal antibody with differential reactivity for strains of human cytomegalovirus. J Med Virol 29: 139–145.

Britt WJ, Vugler L. 1987. Structural and immunological characterisation of the intracellular forms of an abundant 68000 M_r human cytomegalovirus protein. J Gen Virol 68:1897–1907.

Chandler SH, McDougall JK. 1986. Comparison of restriction site polymorphisms among clinical isolates and laboratory strains of human cytomegalovirus. J Gen Virol 67:2179–2192.

Charpentier B, Michelson S, Hiesse C, Martin B, Fries D. 1987. Immunite cellulaire T anti-cytomegalovirus et restriction HLA chez le transplante renal. Ann Med Interne (Paris) 138:334–337.

Dal Monte P, Bessia C, Ripalti A, Landini MP, Topilko A, Plachter B, Virelizier JL, Michelson S. 1996. Stably expressed antisense RNA to cytomegalovirus UL83 inhibits viral replication. J Virol 70: 2086–2094.

Gallina A, Percivalle E, Simoncini L, Revello MG, Gernal G, Milanesi G. 1996. Human cytomegalovirus pp65 lower matrix phosphoprotein harbours two transplantable nuclear localisation signals. J Gen Virol 77:1151–1157.

Gerna G, Revello MG, Percivalle E, Morini S. 1992. Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenaemia. J Clin Microbiol 30:1232–1237.

Gilbert MJ, Riddel SR, Plachter B, Greenberg PD. 1996. Cytomegalovirus selectively blocks antigen processing and presentation of its immediate-early gene product. Nature 383:720–722.

Grefte JMM, van der Gun BTF, Schmolke S, van der Giessen M, van Son WJ, Plachter B, Jahn G, The TH. 1992. The lower matrix protein pp65 is the principal viral antigen present in peripheral blood leukocytes during an active cytomegalovirus infection. J Gen Virol 73:2923–2932.

Klages S, Ruger B, Jahn G. 1989. Multiplicity dependent expression of the predominant phosphoprotein pp65 of human cytomegalovirus. Virus Res 12:159–168.

La Fauci G, Sapienza VJ, Chen CJ, Wisniewski HM, Kim KS. 1994. Expression of the human cytomegalovirus 65k tegument phosphoprotein in insect cells by baculovirus vectors. J Gen Virol 75:189–192.

Landini MP, Guan MX, Jahn G, Lindenmaier W, Mach M, Ripalti A, Necker A, Lazzarotto T, Plachter B. 1990. Large scale screening of human sera with cytomegalovirus recombinant antigens. J Clin Microbiol 28:1375–79.

Landini M, Ripalti A, Sra K, Pouletty P. 1991. Human cytomegalovirus structural proteins: immune reaction against pp150 synthetic peptides. J Clin Microbiol 29:1868–1872.

Naumov NV, Mondelli M, Alexander GM, Tedder RS, Eddleston WF, Williams R. 1984. Relationship between expression of hepatitis B virus antigens in isolated hepatocytes and autologous lymphocyte cytotoxicity in patients with chronic hepatitis B virus infection. Hepatology 4:63–68.

Ohlin M, Plachter B, Sundqvist V, Steenbakkers PGA, Middeldorp JM, Borrebaeck CAK. 1995. Human antibody reactivity against the lower matrix protein (pp65) produced by cytomegalovirus. Clin Lab Diagn Immunol 2:325–329.

Ohlin M, Sundqvist VA, Gilljam G, Ruden U, Gombert FO, Wahren B,

Borrebaek CAK. 1991. Characterisation of human monoclonal antibodies directed against the pp65-kD matrix antigen of human cytomegalovirus. Clin Exp Immunol 84:508–513.

Pande H, Campo K, Tanamachi B, Zaia JA. 1991. Human cytomegalovirus strain Towne pp65 gene: nucleotide sequence and expression in Escherichia coli. Virology 182:220–228.

Pande H, Lee TD, Churhcill MA, Zaia JA. 1990. Structural analysis of a 64-kDa major structural protein of human cytomegalovirus (Towne): identification of a phosphorylation site and comparison to pp65 of HCMV (AD169). Virology 178:6–14.

Plachter B, Klages S, Hagelmann S, Britt W, Landini MP, Jahn G. 1990. Prokaryotic expression of phosphorylated tegument protein pp65 of human cytomegalovirus and application of recombinant peptides for immunoblot analysis. J Clin Microbiol 28:1229–1235.

Riddell SR, Greenberg PD. 1997. T cell therapy of human CMV and EBV infection in immunocompromised hosts. Med Virol 7:181–192.

Roby C, Gibson W. 1986. Characterisation of phosphoproteins and protein kinase activity of virions, noninfectious enveloped particles, and dense bodies of human cytomegalovirus. J Virol 59:714–727.

Ruger B, Klages S, Walla B, Albrecht J, Fleckenstein B, Tomlinson P, Barrell B. 1987. Primary structure and transcription of the genes coding for the two virion phosphoproteins pp65 and pp71 of human cytomegalovirus. J Virol 61:446–453.

St. George K, Rinaldo CR. 1997. Comparison of commercially available antibody reagents for the cytomegalovirus pp65 antigenemia assay. Clin Diagn Virol 7:147–152.

Schmolke S, Drescher P, Jahn G, Plachter B. 1995. Nuclear targeting of the tegument protein pp65 (UL83) of human cytomegalovirus: an unusual bipartite nuclear localisation signal functions with other portions of the protein to mediate its efficient nuclear transport. J Virol 69:1071–1078.

Schmolke S, Kern H, Drescher P, Jahn G, Plachter B. 1995. The dominant phosphoprotein pp65 (UL83) of human cytomegalovirus is dispensable for growth in cell culture. J Virol 69:5959–5968.

Sharma AK, Taylor JD, Tong W, Brown MW, Sells RA, Bakran A. 1997. Utility of the pp65 direct antigenemia test in the diagnosis of cytomegalovirus (CMV) in renal transplant recipients. Transplant Proc 29:799.

Somogyi T, Michelson S, Masse MJ. 1990. Genomic location of a human cytomegalovirus protein with protein kinase activity (PK68). Virology 174:276–285.

The TH, van den Berg AP, Harmsen MC, van der Bij W, van son WJ. 1995. The cytomegalovirus antigenemia assay: a plea for standardization. Scand J Infect Dis 99:25–29.

van Zanten J, Harmsen M, van der Giessen M, van der Bij W, Prop J, de Leij L, The TH. 1995. Humoral immune response against human cytomegalovirus (HCMV)-specific proteins after HCMV infection in lung transplantation as detected with recombinant and naturally occurring proteins. Clin Diagn Lab Immunol 2:214–218.

Wills MR, Carmichael AJ, Mynard K, Jin X, Weeks MP, Plachter B, Sissons P. 1996. The human cytotoxic T-lymphocyte (CTL) response to cytomegalovirus is dominated by structural proteins pp65: frequency, specificity, and T-cell receptor usage of pp65-specific CTL. J Virol 70:7569–7579.